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14. ABSTRACT: The Showcase for Biotechnology 2005 conference brought together scientists and medical professionals from across the country and Europe to present and discuss their research and clinical findings in all phases of biomolecular research including DNA sequencing, genotyping, expressional analysis, protein separation and identification, and biomedical informatics analysis.

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INTRODUCTION

The Windber Research Institute is a private, nonprofit biomedical research institute that, through a unique partnership with Walter Reed Army Medical Center, is focused on the issues of women's health, cardiovascular disease and the process of aging that adversely affect the military and civilian populations of this country. Its mission is to make translational medicine recognize the need to move from its bedside to the bench as well as from the bench to the bedside by optimizing biomedical systems of the future. The genomics, proteomics, biomedical informatics and data fusion communities have a wealth of experience in producing mining and using information generated within their respective domains. Although these domains are largely separate and distinct, medicine will soon be evolving to incorporate the types of data that currently exist only in distinct domains, as well as data in the laboratories of molecular biologists, geneticists, cell biologists, immunologists, and computer scientists. The successful movement of genomics and proteomics information into the clinic, however, will only be significantly accelerated when platforms are standardized and bioinformatics are seamlessly linked to medical informatics. This can be achieved through public and private collaboration among clinicians, scientists and engineers, to bridge existing gaps among those disciplines, improving and standardizing the methods used in data collection, and efficiently managing the storage, analysis and retrieval of the massive amount of data generated from new large-scale experimental techniques such as microarray gene expression profiling, 2 dimensional gel electrophoresis, multi-dimensional chromatography and mass spectrometry. The challenges associated with the generation of massive amounts of molecular information using automated systems and linking genotype and phenotype information offers interesting opportunities for public/private collaboration. Making this linkage is a key step in linking biologically and clinically useful information, elucidating biochemical pathways, stratifying disease, understanding the mechanism of known drugs, discovering new drugs and moving scientific discoveries into the clinic fast.

Windber Professional Services, Inc. (WPS) is the lead organization that markets the services and products offered by the Windber Research Institute (WRI). WPS, through its partnership with WRI, provides commercial access to genomic and proteomic datasets and advanced methods of data analysis. WPS also serves as a catalyst for regional economic growth by its services in the areas of operations management, marketing, campus planning, property management, business and company development, information management, and accounting.

BODY

The Showcase for Biotechnology 2005 conference brought together scientists and medical professionals from across the country and Europe to present and discuss their research and clinical findings in all phases of biomolecular research including DNA sequencing, genotyping, expressional analysis, protein separation and identification, and biomedical informatics analysis. The presentations and abstracts with presenter biographies follow:

KEY RESEARCH ACCOMPLISHMENTS

N/A

Individualized Genomic Stress Induction Signature Reversal With Recreational Music-Making

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Dr. Bittman presents a concise overview of his latest research that focuses on reversing the biological impact of stress at the DNA level. He discusses a novel recreational music-making program that was used in combination with a new strategy for peripheral blood gene expression analysis to assess individualized genomic stress induction signatures. Dr. Bittman describes a unique two-phase protocol

for inducing and subsequently ameliorating stress, and the surprising results that surfaced through extensive statistical analysis. Modulation of individualized genomic stress induction signatures in peripheral blood is presented as a new opportunity for elucidating the dynamics of the human stress response in the context of potential clinical utilization.

Identification of Molecular Changes Associated with Breast Tumor Phenotype in African American Women.

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The incidence of breast cancer varies among different ethnic populations. The overall incidence of breast cancer is highest among Caucasian women (CW); however, mortality is higher and survival rates lower among African American women (AAW). These conflicting data are frequently attributed to differences in socioeconomic status and disparities in health care access among the two groups. However, tumor biology also varies among the two populations. Specifically, tumors in AAW are larger, more aggressive, and poorly differentiated. In addition, they have high grade nuclear atypia, greater lymph node involvement, and are more frequently estrogen receptor negative. These differing tumor characteristics suggest that a biological component(s) is responsible for the worse prognosis among AAW with breast cancer.

Due to the striking similarity of breast tumors from AAW to those from women with inherited mutations in BRCA1, our preliminary work focused on BRCA1. However, no significant associations between BRCA1 and the aggressive tumor phenotype in AAW were found. Due to the complexity and multigenic nature of breast cancer, we felt that a global approach would more effectively identify genes responsible for breast tumor development and the poor clinical outcomes in AAW. Presently, we are using microarray analysis to determine if gene expression differences exist in normal breast tissue that predispose AAW to a more aggressive form of breast cancer.

We collected normal breast tissue (reductive mammoplasty and/or disease-free biopsy specimens) from 13 pairs of age-matched AAW and CW. RNA was isolated, amplified and hybridized to the CodeLink Human Whole Genome Bioarray, representing over 45,000 transcripts. Mann-Whitney testing was performed to identify differential gene expression between AAW and CW ($p < 0.01$). Differentially expressed genes were then categorized according to their gene ontology.

Over 500 differentially expressed genes were identified. When these genes were mapped to their biological process ontology, we found that genes involved in cell proliferation were expressed at higher levels while cell adhesion genes were expressed at lower levels in normal breast tissue from AAW compared to CW. These results suggest that breast cells in AAW may divide more rapidly than those in CW while loss of contact inhibition mediated by cell adhesion molecules may lead to unrestrained cell growth in breast tissue from AAW. The resulting increase in cell proliferation may serve as an oncogenic stimulus and/or prematurely age the breast tissue in AAW.

The Einstein Center for Urban Health Policy and Research: Using Mixed Methodologies to Study Health-Related Beliefs, Practices, and Outcomes

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Health disparities are best understood and addressed using mixed methodologies that investigate various levels of inquiry, including the genomic the personal, the community, the larger societal, and beyond. The overarching theme of the Einstein Center is to investigate and address social, economic, cultural and environmental factors essential in understanding health. This includes the experience of illness; how people think about and cope with disease, and specific health outcomes, including health disparities. This presentation will focus on the use of mixed methodologies, specifically the use of qualitative and quantitative approaches. The qualitative paradigm, which is less familiar to researchers, is subjective, generative and naturalistic and requires direct subject contact, usually interviewing or observation. Qualitative approaches are critical to uncovering factors missed by structured approaches. When combined, these mixed methodologies add value to research findings by providing patient-centered, evidenced-based results with which to improve organization and delivery of health care to patients; to better understand differences in health outcomes, and to develop practical and culturally sensitive interventions to address remediable health disparities. Examples from our research will be presented.

Dealing With Uncertainty in the Assessment of Diagnostic and Prognostic Factors in Cancer

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Accurate and reliable decision making in cancer diagnosis and prognosis can help in the planning of suitable surgery and therapy, and generally, improve patient management through different stages of the disease. In recent years, several diagnostic and prognostic markers including cytometric and genetic factors have been used as indicators of disease progression in cancer. However, the rapid increase in the discovery of novel markers resulting from the developments in biomedical technology, has dictated the need for developing reliable methods for stratifying clinically significant markers where complex and nonlinear interactions between these markers naturally exist. It has been shown that whichever computational approach, even the most sophisticated computational tool, is used for the assessment of diagnostic and prognostic factors, each method can identify different sets of markers as the most associated with the disease. This obviously leads confusion over the outcome of the analysis. This issue needs addressing as such study relying on only outcome of a single approach may therefore result in misleading information, and subsequently affecting a medical professional's decision over his/her patient as well as national framework for cancer patient management being established. In this paper, new methodology where appropriate computational intelligence tools, namely fuzzy logic and hybrid neuro-fuzzy rule-based systems, have been incorporated will be presented to show how uncertainty in the assessment of these markers has been dealt with and how reliability of selecting clinically significant markers be increased. The results obtained through the analysis of the data collected for breast, prostate and collateral cancers will be demonstrated.

Biomedical Informatics Research and Development at Windber Research Institute

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The biomedical informatics infrastructure development at Windber research institute continues. In this presentation, I will discuss about our Phase III development of the data warehouse which is of a patient-centric modular structure. I will also give an overview of several research and development projects in the fields of quality assurance, data analysis, and data mining across the clinical, genomic, and proteomic platforms. These developments are laying the foundation for our integrative research using the systems approach.

Future Direction of the Human Genome Project

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Genomics and the Future of Medicine: Beyond the Human Genome Sequence

The Human Genome Project's recent completion of the human genome sequence represents a spectacular scientific achievement of historic proportions. It also signifies a critical transition, as this new, powerful foundation of genetic information is used by researchers and clinicians to tackle complex problems in human biology and disease. The next phase of genomics research will focus on connecting genomic data and technologies to biology, to health, and to society. In the coming decades, it will likely also change biomedical research and the practice of medicine in profound ways.

When Good Data Goes Bad: Thoughts Regarding Data Quality

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On an intellectual plane scientific research aspires to a greater understanding of the natural world. On the other hand, the business of scientific research, motivated by financial bottom lines, seeks scientific knowledge for profit. These two goals are sometimes conflicted as pressures exerted by financial considerations demand ever greater data volumes, faster throughput and more efficient analyses in order to bring novel products to market. To this end, pure science and the business of science research have both benefited greatly in last decade by the development of fresh, innovative high throughput laboratory technologies, many with which are only practical because of dazzling advances in computer technologies. The result has been the continual creation of enormous data warehouses. The dilemma that accompanies massive data acquisition and processing is that of data quality. This presentation is an

overview of data quality issues. Guidelines are offered for processes and procedures which may be implemented in Quality Control and Quality Assurance programs.

HER2 Gene Amplification is a Marker for Global Genomic Instability

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From the Clinical Breast Care Project, Windber Research Institute (REE, DLE, BD, HLP) and Walter Reed Army Medical Center (JAH, CDS)

BACKGROUND Genomic alterations of the proto-oncogene c-erbB-2 (HER-2/neu) are associated with poor prognosis in patients with breast cancer. While HER-2 status has predictive value for the efficacy of different therapies, its long-term prognostic relevance remains controversial. Because some patients with HER-2 gene amplification do not exhibit corresponding over-expression of the HER-2 protein, it is unclear how genomic alterations correlate with clinical response. To assess the broader genomic implications of structural changes at the HER-2 locus, we investigated relationships between global genomic instability and HER-2 status in patients with invasive breast cancer.

METHODS DNA was extracted after laser microdissection from 107 paraffin-embedded invasive breast tumor specimens, 28% of which had HER-2 amplification determined by the PathVysion® fluorescence in situ hybridization (FISH) assay. Referent DNA was extracted from blood, disease-free skin, or negative lymph node samples. Allelic imbalance (AI) was assessed using a panel of microsatellite markers representing 26 chromosomal regions commonly deleted in breast cancer. The threshold normalized peak height ratio to detect AI was set at 0.35 and t-tests were used to investigate relationships between genomic instability and HER-2 status.
RESULTS Overall levels of AI did not differ significantly in patients stratified by stage, lymph node, or menopausal/hormonal status. The frequency of AI was significantly higher ($P<0.05$) however, in patients with HER-2 amplification (29%) compared to those without (21%). When individual chromosomal regions were examined, samples with HER-2 amplification showed significantly higher levels of AI ($P<0.0001$) at chromosome 17q21 (located <5 Mb centromeric to the HER-2 locus and includes BRCA1), as well as chromosomes 6q15, 18q21 and 22q12.3 ($P<0.05$).
CONCLUSIONS HER-2 gene amplification may serve as a marker of global genomic instability. Concurrent genetic alterations in other regions of the genome may contribute to poor prognosis and influence associations between HER-2 status and outcomes of adjuvant chemotherapy and Herceptin® treatment. The addition of molecular profiling for AI may thus enhance treatment decisions for individuals with HER-2 amplified breast cancer.

Novel Ion Channel Genes: From Fertility to Respiratory Rhythm Control

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Ion channels control essentially every aspect of our physiological responses: from excitation of muscle and neurons to learning, memory and gene expression. The about 150 ion channel genes in human genome are important pharmaceutical targets. We have been studying ion channel genes involved in calcium signaling and cell excitability in mammals. Using a bioinformatics approach, we have discovered

several novel ion channels expressed in wide range of tissues. One such ion channel family (CatSper1-4) is exclusively expressed in testis and sperm tail. Using a mouse model, we found that a CatSper protein is exclusively required for male fertility. At the cellular level, the channel protein is essential for calcium entry into sperm tail, which in turn drives the hyperactivated sperm motility required for sperm to penetrate eggs during fertilization. Thus, CatSper channel family represents ideal targets for male contraceptive development. Other novel ion channels we discovered are expressed in kidney, heart and brain. One such a channel gene is essential for the generation of the neuronal rhythm controlling breathing. Animals with genetic defect in this gene show severely disrupted respiratory rhythm and die shortly after birth. This novel ion channel may be a good target for drugs to modulate neuronal excitability.

Correlation Of Gene Expression Profiles Of Breast Cancer Patients With Tumor Detectability By Mammographic Screening

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Introduction: Mammography screening is one of the most effective and widely used tools for the early detection of breast cancer. However, less than 30% of breast cancer cases were detected by this method. Joensuu et al published results (JAMA Sep 2004) indicating that patients had an improved 10-year disease-free survival if their cancerous tumors were first detected by mammographic screening as opposed to clinical- or breast-self exam. We compared gene expression profiles of patients with mammographically versus non-mammographically detected breast cancers, in an attempt to identify potential molecular markers that might be associated with the Joensuu findings.
Material and Methods: We identified 49 patients with tumors from our CBCP microarray database that fit the criteria (unilateral invasive breast carcinoma, greater than 1 cm in size and node-negative) used by Joensuu et al. Blood RNA from these 49 patients were isolated and used for microarray analysis using CodeLink UniSet Human 20K Bioarrays. Among these patients, 22 had tumors that were detectable by mammography screening (MMG) while 27 were not detectable by this method (NONMMG). The raw signal intensities from the microarrays were imported into GeneSpring data analysis software (Agilent Technologies) and normalized using global normalization. Differentially expressed genes were identified based on a fold-change and used for hierarchical clustering. Among these 49 patients, 35 are Caucasian, 7 African Americans, 2 Asian Americans, 4 Hispania, and 1 Native American. We studied only those 35 Caucasian patients to avoid potential bias caused by the different ethnicities.
Results: We identified 11 genes that had greater than 1.7 fold differences in expression between MMG and NONMMG samples. Using these 11 genes, we were not able to separate all 49 samples into two cluster groups. However, when we studied only those 35 Caucasian patients, we were able to identify 8 genes and used them to separate these 35 samples into two groups. One cluster was composed of 15 MMG samples and 4 NONMMG samples while the other group contained 5 MMG samples and 11 NONMMG samples. Further stratification on the basis of Lymph Node positive and Lymph Node negative did not provide any improvement in clustering because of small sample size.
Discussion: Microarray results relating to expression of 19982 genes in white blood cells from cancer patients could not identify genes that were suitable for clustering analysis on the original samples. After using a subset that contained only Caucasian samples, we were able to identify genes that could be used in hierarchical clustering with a degree of sensitivity at 75% and specificity at 73%. We further stratified these samples based on Lymph Node status. Used only Lymph Node positive samples, we were able to identify genes that can be used in hierarchical clustering. But due to the small sample size, degree of sensitivity and specificity might not be meaningful. These results indicated that with proper experiment design and classification of samples, molecular markers can be identified using microarray technology and the potential use of microarray technology as diagnosis tool.

An Application of Array CGH Technology to Genomic Studies

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We will discuss the potential that exists, through the application of aCGH technology, to correlate genomic copy number aberrations to clinical outcomes. Statistical algorithms and software that we have developed will be presented in the context of preliminary results associated with several on going studies. Sample size requirements for the design of aCGH studies will also be discussed. A novel method for the estimation of the False Discovery Rate in aCGH experiments will be presented.

The Role of Boron in Prostate Cancer

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Susan L. Meacham[1,3], Kyler E. Elwell[2] and Stephen W. Carper[2,3]

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[3]UNLV Cancer Research Center, University of Nevada Las Vegas

Dietary boron intakes can vary widely depending on the types of foods eaten, dietary supplements consumed and the source of drinking water. Currently, there are no recommendations for minimum or recommended daily dietary intakes of boron. The total boron content of the average daily diets of selected US populations reporting intakes range from 548 µg for toddlers (2 yrs of age) to 890 µg for males (25-30 yrs of age). Contributions to daily boron intakes from common dietary supplements may be adding as little as 0.15 µg or as much as 40 mg to the daily boron intakes from foods and beverages. The inability to identify the biochemical mechanisms for boron in normal plant and human metabolism have elucidated investigators for decades and prohibited boron from being classified as an essential nutrient for humans. However, recent epidemiological studies have shown an inverse relationship between diets high in boron and prostate cancer. To begin studying the molecular mechanism relating high dietary intakes to lower incidences of prostate cancer, three prostate cancer cell lines were cultured in the presence of 1 mM boric acid, the predominant form of boron in human plasma. Two cell lines (PC3 and LNCaP) showed no effect while growth of the DU-145 prostate cancer cell line was completely inhibited. There were no differences in cell cycle kinetics analyses between the boric acid treated, growth inhibited prostate cells and the untreated, control DU-145 cells.

Joan Schank, MPA – PTEI – Presentation
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Tissue engineering is at the forefront of modern medicine and the Pittsburgh Tissue Engineering Initiative (PTEI) has been at the forefront of tissue engineering for nearly a decade. The mission of PTEI is to improve the health of individuals by establishing the region as an internationally recognized center of excellence in research, education, and commercial development for the advancement of tissue-related medical therapies.

In addition to participating in the Windber Research Institute's Showcase for Biotechnology 2005 as exhibitor and sponsor, PTEI is pleased to feature a component of one of our many educational and workforce development programs, *The Adventures in Biotechnology Program*. On Monday, August 15, 2005 students from Windber High School -- participants in the *Adventures in Biotechnology Program* -- who have formed a working partnership with researchers and leaders of the Windber Research Institute, will present their work to Congressman John P. Murtha.

Cardiac CT: A New Method to Assess Coronary Artery Disease

Thomas J. Brady, M.D.

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The objectives of his presentation are:
1) Understanding the principles of data acquisition and processing.
2) Understand image interpretation
3) Review CT in coronary artery disease and other applications

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The objectives of his presentation are:
1) The value of PET imaging in the evaluation of coronary artery disease
2) The role of multidetector CT in the evaluation of coronary artery disease
3) The value of combined PET and CT imaging in coronary artery disease

Changing Medical Management with FDG PET

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Course Description:
This lecture is designed to update participants on the use of PET/CT Imaging to enhance patient care in the clinical setting.

The objectives of his presentation are:
1.) Discuss the use of PET imaging in the clinical setting.
2.) Describe when PET imaging is beneficial to the diagnosis and treatment of certain disease states.

PET/CT State of the Art – Clinical Applications

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The objectives of his presentation are:
1.) Identify the technical aspects of patient preparation and imaging protocols of PET/CT.
2.) Role of PET/CT for diagnosis, staging and restaging in oncology, assess response to therapy.
3.) Explain the benefit of using PET/CT for radiation therapy planning.
4.) Role of PET/CT in cardiac imaging and molecular cardiology

PET Imaging Technology and Applications in Cardiology

Phil Vernon
Global Research Manager for PET/CT
GE Healthcare Technologies

PET/CT provides a unique capability to provide information about the anatomy and function of the heart. PET has been used for many years to image and measure the distribution of myocardial perfusion, both and rest and under stress. The process is similar the that commonly used in gamma camera imaging, except that PET is less susceptible to errors due to attenuation and the spatial resolution is better. Despite these advantages, PET imaging of myocardial perfusion has not been widely used. The principal reason is that access to PET scanners has been difficult. Over the last four years there has been a dramatic increase in the availability of PET scanning, so the situation is now changing.

FDG PET imaging provides additional data. If there is a "fixed" perfusion defect, FDG can distinguish between infarction and "hibernating myocardium", providing guidance on whether revascularization is likely to be beneficial.

When modern CT is combined with PET, the system is capable of calcium scoring and of angiography. CT can therefore add valuable information about the history of coronary artery disease, demonstrate the gross coronary anatomy of the vessels, and demonstrate focal narrowings of the main vessels.

All of these studies can, in principal, be carried out in a single examination, and the data is inherently registered. The question now is, how useful is this technology in the management of coronary artery

disease? There are also some significant problems that require resolution if these combined tests are to be used widely.

The attendee will take away:
an outline of the capabilities of PET/CT in cardiology,
the advantages and disadvantages of PET/CT compared to competing technology
an understanding of the outstanding problems in performing combination testing
information about future improvements of PET/CT systems,
an understanding of the questions to be answered in evaluating the technology.

Stochastic Simulation of Biochemical Systems

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Modeling and simulation promise to revolutionize the way biologists work, in much the same way that engineers use computer-aided design tools to assure properties of systems that they build. However, biological systems involving genetic reactions can be extremely large and complex, and often contain certain species that occur in small quantities and others that occur in large quantities, leading to a difficulty in modeling and simulation. Small populations inhibit the usefulness of utilizing differential equations to represent the system, while the large populations cause stochastic discrete event simulation to become computationally intensive.

In this talk, we present an algorithmic approach for the dynamic partitioning and stochastic hybrid simulation of biological systems, building on results that have been used in more traditional engineering fields, but adapting and inventing new approaches as needed. The hybrid simulation algorithm we present uses a Poisson approximation for discrete event generation and ordinary differential equations to model continuous behavior. The populations are dynamically partitioned so that some populations are simulated in a discrete stochastic fashion, while others are simulated by continuous differential equations, and this partition between discrete and continuous behavior is updated. In addition to presenting the algorithm itself, we describe its place in a discrete event modeling and simulation tool known as Möbius, which is finding growing use in biological applications.

PubChem and NIH's Molecular Libraries Initiative.

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PubChem is a new online information resource from the National Center for Biotechnology Information, NCBI. The system provides information on the biological properties and activities of chemical substances.

Following the sequence-deposition model followed by GenBank, PubChem's content is derived from user depositions of chemical structure and bioassay data, including new bioassay data from NIH's recently

funded Molecular Libraries Screening Center Network. PubChem's retrieval system supports searches based on chemical names and chemical structure, as well as searches based on bioassay descriptions and quantitative bioassay results. PubChem furthermore provides links to depositor sites, for further information, as well as links to other NCBI resources such as the PubMed literature database and Entrez's protein 3D structure database.

Use of Bayesian Networks and Machine Learning to Map Complex Diagnoses to Outcomes

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We modeled a set of pathologically characterized breast cancer specimens with a machine learning Bayesian network to predict recurrence of disease. 75 HER2 by immunohistochemical (IHC) negative breast cancer specimens with one to eleven years of clinical follow-up were collected at Sharp Memorial Hospital in San Diego. The specimens were characterized by histopathologic features, prognostic and predictive markers, and knowledge of recurrent disease. Data analysis was conducted using a machine learning Bayesian Belief Network. The Bayesian networks used in this paper were developed using the DecisionQ FasterAnalytics software. Our model showed that Combined Nottingham Histologic Grade (CNHG) , tumor stage, and tumor type were significant predictors of recurrent disease. In cross validation testing of the model, area under the curve for recurrent disease prediction was 76.9%, area under the curve for non-recurrent disease prediction was 75.8%, positive predictive value was 73.3% and negative predictive value was 80.7%. Using the DecisionQ software, we have been able to successfully model common pathology descriptors and outcomes data using a machine learning Bayesian network. When cross validated, the network shows strong predictive power to determine recurrence of disease using common pathology descriptors.

Bayesian and Non-Bayesian Computational Analyses of Heterogeneity in Breast Disease, A Case Study in Datamining Clinical Records for Disease Hypothesis Generation

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A systematic and quantitative study of heterogeneity in breast tissue would enable us to characterize the complex pathologic associations within and between breast disease patients. Our analysis focuses on patterns in pathology diagnoses, and our dataset is drawn from 1020 pathology reports. Bayesian and non-Bayesian analyses run on this dataset are yielding intriguing patterns in diagnosis co-occurrence in CBCP patients.

Breast Cancer Risk Models – Integrating Molecular, Imaging, and Patient Diagnostics to Improve Disease Management

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Purpose:
Develop technologies for risk and prognostic modeling of breast cancer using an integrated dataset of digital mammography, microarray, proteomic, and patient history data.
Clinical Question:
How does one optimize the use of readily accessible technologies to more accurately evaluate breast cancer risk and prognosis in women with dense breasts?
Materials and Methods:
We will combine clinical data composed of breast density measurements, proteomic data on blood serum, patient history questionnaire data, microarray data on Peripheral Blood Mononuclear Cells (PBMC) from high risk women with no prior history of breast cancer. We will evaluate the hypothesis that predictive models combining such data can provide higher sensitivity and/or specificity for pathology outcome than the standard Gail Model against an outcome of abnormal pathology in which the biopsy was warranted. Using this model we hope to provide a tool that can reduce unnecessary biopsies.
We further hypothesize that women can be stratified by such data into classes which demonstrate varying sensitivity and/or specificity of mammography readings with respect to pathology abnormality as above. The goal is to give information prior to mammogram to improve the reading and affect screening choices for women with higher predictive scores.

Towards Mechanistic Modeling and Systematic Analysis of Human Cells for Cancer Prognosis and Treatment.

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Our collective understanding of the control mechanism of the human cell cycle has increased steadily in the past decade. We are at a point where we can begin to construct molecular-level cell models that use genomic and proteomic measurements to predict the development of the hallmarks of cancer: self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. Recent studies have indicated that the simultaneous consideration of molecular alterations contributing to the hallmarks of cancer might provide useful prognostic and therapeutic information.

We propose to build a model of cancer progression based upon the cell cycle control mechanism and validate its predictions based on known biomarkers that are functionally categorized via the hallmarks of cancer. This model will require hybrid simulations that combine stochastic events in genetic modifications with the continuous processes of tumor development and growth that result from acquisition of hallmarks. Design optimization techniques, along with abundant computing power will be employed to identify

stochastic model parameters and continuous model rate constants that best fit the observed lab measurements.

The end result will be a mechanistic and statistical approach to understanding cancer progression, providing researchers with a method to generate and test hypotheses regarding the pathways associated with cancer, and ultimately a linkage from lab measurements to clinical insights. In this talk, we will present the rationale for our proposed approach, outline our work plan for deriving preliminary data, and solicit feedback.

Silicon Research Center (GSRC) and on the Executive Committees of both the GSRC and the MARCO/DARPA Center for Circuit and System Solutions (C2S2) (http://fcrp.org)

His contributions have been recognized with the 1993 Eta Kappa Nu Outstanding Young Electrical Engineer Award, the 1994 Xerox Award for Faculty Research, the 1994 University Scholar Award of the University of Illinois, the 1997 Eta Kappa Nu Holmes MacDonald Outstanding Teaching Award, the 1998 ACM SigArch Maurice Wilkes Award, the 1999 ACM Grace M. Hopper Award, the 2001 Tau Beta Pi Daniel C. Drucker Eminent Faculty Award, and the 2002 ComputerWorld Honors Archive Medal. He is a fellow of IEEE and of the ACM.

The Role of Diagnostic Testing in the Treatment of Colorectal Cancer

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Colorectal cancer (CRC) is the second leading cause of cancer death in the United States. Several agents are now available for the systemic therapy of patients with colorectal cancer including; fluorpyrimidines, irinotecan, oxaliplatin, bevacizumab and cetuximab. In metastatic disease and in the adjuvant setting, the addition of other agents to a 5-Fluorouracil (5-FU) backbone results in improved survival. Two approaches to build on the recent advances in systemic therapy of colorectal cancer are being pursued: (1) exploration of alternative combinations of available agents and (2) identification of patient tumor characteristics (pharmacogenetics and pharmacogenomics) that will be predictive of response and toxicity.

CME Mammography

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1) Digital Mammography – Current Capabilities and Limitations
2) Medicolegal Aspects of Breast Cancer – Challenges for the Whole Breast Physician Team

New Multi-Dimensional Strategies For Identifying Low Abundance Proteins In Serum And Plasma For Disease Biomarker Discovery.

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The identification of low abundance proteins in human plasma and serum that are putative disease biomarkers is problematic due to extreme sample complexity, a wide range of protein concentrations, and extensive biological variation. The biggest problem is that a handful of serum proteins present at mg/ml levels severely limit sample loading capacities for most separation methods, thereby making detection of potential disease biomarkers in the ng/ml to pg/ml ranges extremely difficult. To enable detection of these low abundance proteins, reduction of proteome complexity by multiple tandem dimensions of protein prefractionation is essential. We recently developed a powerful 4-D protein profiling strategy for serum and plasma that includes three orthogonal protein separations: major protein immunodepletion, microscale solution isoelectrofocusing (MicroSol-IEF), and 1-D SDS PAGE. The result is a 2-dimensional array of pixels or gel slices that is informationally equivalent to a low resolution 2-D gel, since each pixel in the array contains a group of proteins with a known pI and molecular weight range. Each pixel is then digested with trypsin followed by nanocapillary reversed phase tryptic peptide separation prior to tandem mass spectrometry analysis. When human serum was analyzed, more than 2,700 proteins spanning up to nine-orders-of-magnitude were identified using HUPO criteria for high confidence assignments. More importantly, a substantial number of low abundance proteins (< 100 ng/ml to pg/ml range) were identified. Although this performance is already substantially superior to older alternative methods, our 4-D strategy is currently being further optimized to obtain more comprehensive detection of low abundance proteins, particularly expanded coverage of proteins in the pg/ml range. This method is also being applied to cancer biomarker discovery in mouse model systems and human serum samples.

Chromatography of Difficult and Water-Insoluble Proteins

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Histones, integral membrane proteins, and pathogenic prions are examples of proteins that either aggregate in or are not soluble in water. This interferes with attempts to perform assays and limits progress in proteomics and biochemistry in general. Solubilizing agents such as detergents or urea interfere with chromatography and are incompatible with mass spectroscopy and other instrumental methods. Organic solvents can frequently be used as alternative additives in the mobile phase with the elimination of some or all of these problems. The quality of the resulting chromatography can be remarkably good, even for difficult proteins like the ones listed above. This lecture will address the benefits of organic solvents in such applications, for both individual proteins and complex extracts. Alternative approaches to proteomics will be discussed in connection with these methods.

Genetic Analysis of Complex Disese: The SOP^3 Web Server for Design of Oligonucleotide Primer Trios

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Design of locus specific primers for use during genetic analysis requires combining information from multiple sources. This is a time consuming process when validating assays for large numbers of polymorphisms. Data warehousing of publicly available information, coupled with software applications for optimizing the generation of locus specific primers, can increase the efficiency of assay development. Selection of Oligonucleotide Primers for PCR and Pyrosequencing (SOP^3) software allows user directed queries of warehoused data collected from existing genome projects, automation of processes such as collection of genomic sequence, identification of single nucleotide polymorphism (SNP), and incorporation of locus-associated functional information. The program accepts as input either gene locus symbols, SNP reference sequence numbers, or chromosomal physical location. The program allows primer trio design for genetic analysis of human as well as mouse polymorphisms. For human polymorphisms, SOP^3 incorporates haplotype, ethnicity, and validation attributes. The output is a list of oligonucleotide primers recommended for use in sequence-based genotyping to be used in evaluating the inheritance patterns of SNP markers. Availability of a dataset of validated primer trios furnishes a unique resource for proven assay conditions. SOP^3 is available at URL http://imgen.ccbb.pitt.edu/sop3v2. This work was supported by grants U19-AI056374-01 Autoimmunity Centers of Excellence, RO1DK24021 from the NIH, and ERHS #00021010 from the DOD.

Proteomic Poetry: A Pattern-Based Approach to Biomarker Discovery

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The nature of disease (and health) is inherently complex. It is a state, or a multiplicity of states, of an extraordinarily complex biological system. Any study of disease which aims to illuminate causality, detection, diagnosis, treatment and cure must take this complexity into account. Molecular biomarkers, and in particular proteomic biomarkers, have been in use for decades but have recently enjoyed a renaissance due to the development of new methods of detection and profiling, most notably the development of advanced methods of mass spectrometry and electrophoresis and the concomitant development of computational approaches able to cope with vast amounts of data generated experimentally.

The problem with biomarkers is that they are often studied and used individually. The classical cancer markers (e.g. PSA for prostate cancer, CA125 for ovarian cancer) are familiar examples. They are clinically valuable in spite of the fact that their sensitivity and specificity are poor. The traditional approach based on hypothesis-driven discovery of individual biomarkers is being supplanted by a new approach. The use of complex patterns of many proteins as compound biomarkers is becoming feasible due to advances in experimental and computational methods.

Why patterns? Shakespeare wrote his sonnets with words. Each individual word is unremarkable. The specific collections of words, and their spatio-temporal relationships, are unique and beautiful in their ability to express complex thoughts and emotions. There is no keyword of a poem that conveys its significance – the entire *pattern* is required. The expressive power therefore is carried largely in the arrangements of words.

Likewise, we believe that information in high-dimensionality systems like biology and chemistry is carried largely in the *interactions* of descriptors rather than in the descriptors themselves. The descriptive power of a complex pattern of protein biomarkers is vastly greater than that of an individual biomarker, and is more in keeping with the inherent complexity of the biological system it seeks to describe.

Given this thesis, the problem of biomarker discovery can be restated as the couplet of detection (experiment) and enumeration (computational analysis) in a data-driven rather than a hypothesis-driven approach. That is, we seek to profile as many proteins as possible in populations of individuals with and without disease. We then seek to discover patterns, or subsets, of proteins that are maximally informative with respect to the presence or absence of disease. It is preferable to avoid imposing artificial constraints upon this process, either experimentally (for example bias towards a specific subset of proteins) or computationally (for example *a priori* assumptions regarding the number of proteins that may constitute an informative pattern). Thus, the ideal experimental methodology will consist of an approach independent of hypothesis, yet capable of broadly and sensitively surveying proteins within the experimental matrix; e.g. serum or other biological fluid. The ideal computational approach will be capable of assembling, without bias, arbitrary numbers of individual proteins into 'proteomic poems' that have high information content *even if their individual constituents do not*.

This talk will illustrate by way of examples the methods that have been developed and used at Cira to discover and validate proteomic biomarker patterns.

Perifosine, a Novel Alkylphospholipid is an Inhibitor of cPLA$_2$ for Human Cancers.

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Perifosine (NSC 639966) is a new, novel class of orally bioavailable alkylphospholipid compound. Both *in vitro* and *in vivo* several human tumor model systems displayed significant antiproliferative activity by perifosine. It has recently entered phase-I clinical trials because of its better therapeutic index. However, the mechanisms by which perifosine exercise this favorable effect is still unclear. Shortly after exposure of perifosine in different cellular system clearly indicate that cells are undergoing death process. Besides death effect, our laboratory has identified that perifosine could cause cell cycle arrest in a way that correlated with induction of $p21^{WAF1/CIP1}$ in a p53 independent fashion. Structurally perifosine resembles as phospholipids analogue. Here, we also investigated the existence of a biochemical route connecting this $p21^{WAF1/CIP1}$ expression by perifosine that governed natural phospholipids in response to the action of growth factors, reflecting their key pleiotropic role to stimulate cell cycle progression. We demonstrated that the downstream drug effect on induction of $p21^{WAF1/CIP1}$ is mediated via PPAR–γ pathway. We also demonstrated that the cell death effect of perifosine is mediated via PI3K/Akt survival pathway through $cPLA_2$ and Cox2. Interestingly, our data supports that overexpresion of activated form of Akt override at least in part, the cells death effect of perifosine. More interestingly, $cPLA_2$ overexpression completely nullifies perifosine mediated death effect. These findings are consistent with the idea that perifosine causes death to the cells through a pathway involving $cPLA_2$, Akt and Cox2. Our results demonstrate that $cPLA_2$ as an important cellular target of perifosine action linked to Cox2 metabolite. Therefore, as

perifosine down regulate growth factors mediated activation of PI3K/Akt survival pathway that protects cells against death are of clinical interest for single and combined anticancer treatment modalities.

Applying a "Divide and Conquer" Strategy to Proteomic Discovery: IgY Proteom / Partitioning and ProteomeLab PF 2D / Fractionation Applications in Biomarker Discovery.

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A proteome may be a treasure trove for biomarker discovery, however it is clear that such complexity and breadth of dynamic range in protein concentration create a significant challenge for today's analytical technologies. As a result, strategies for simplifying the sample prior to analysis are being developed. In this presentation we propose a partitioning & fractionation strategy which utilizes selective enrichment techniques such as proteome partitioning by affinity capture upstream of multi-dimensional proteome fractionation. We will discuss methods of automated two dimensional proteome fractionation by isoelectric point and hydrophobicity, as well as enrichment techniques for low abundant proteins and techniques for alleviating the masking effect that protein mass fingerprints from high abundance proteins can create.

CME Lecture – Breast Imaging And Ultrasound Applications

Daniel A. Tyskiewicz, RDMS
Clinical Applications Specialist
GE Medical

The purpose of my lecture is to educate the listener on some of the complexities and benefits of Breast Ultrasound. I will start by reviewing the breast anatomy then go into sonographic features of benign and malignant lesions. I will then cover the proper scanning techniques for the sonographer and non-sonographer. There will be an image review and to close I will cover what is on the breast registry.

Poster Sessions

An Analysis Of Data Studying The Prevalence Of Breast And Prostate Cancers In Cambria, Somerset, And Lackawanna Counties In Comparison To Pennsylvania, Including Risk Factors Of Age And Ethnicity.

Windber High School
Toni Boyer, Josh Manculich, Nate Pallo And Kristen Phillips
A Cooperative Program With Windber Area High School And The Windber Research Institute

The objective of this analysis was to investigate the rates of breast and prostate cancers found in Cambria, Somerset and Lackawanna Counties in comparison with each other and Pennsylvania. Also included is a comparison and contrast of in-situ and invasive cancers. In addition, the study included the prevalence of risk factors such as age and race/ethnicity, that have been suggested to affect these populations.

An Analysis Of Data Studying The Prevalence Of Diabetes And Obesity In Cambria, Somerset, And Lackawanna Counties In Comparison To Pennsylvania And The United States Including Risk Factors Of Age And Ethnicity.

Windber High School

Alyssa Diloreto, Kaylee Hollern, Steve Hudak
A Cooperative Program With Windber Area High School And The Windber Research Institute

The objective of this analysis was to investigate the relationship between obesity and diabetes and the rate found in Cambria, Somerset and Lackawanna Counties in comparison with each other, Pennsylvania, and the United States. Also included in the analysis were the prevalence of risk factors such as age and race/ethnicity which have been suggested to affect diabetic and obese populations.

Structure Identification of Unknown Compounds Detected in Mepivacaine Clinical Injection Samples

Luwang Andy Zhu[1], Jennifer Killen[1], Cynthia H Shields[2], Chester Buckenmaier[2], And Qinhua Cindy Ru[1,*]

Local anesthetics are used in a variety of field, austere and combat environments. It is a clinical impression that local anesthetics persistently stored at high temperatures are less effective when used for regional or neuraxial anesthesia[1]. Four kinds of local anesthesia injections including mepivacaine, bupvicaine, tetracaine and ropivacaine have been stored at room temperature, 42 ºC and 57 ºC in the controlled temperature ovens with a recording thermometer, and analyzed by high performance liquid chromatography-ultra visual detector (HPLC-UV) at 15, 30, 45, 60, 90 and 120 days of storage. Every sample has been assayed in triplicate to help reduce measurement error, and the results of HPLC-UV assay did revealed that the long-term high temperature storage of local anesthesia led to the degradation. Besides, the unknown peak generated by the degradation of mepivacaine was detected by HPLC assay, and the structure of this unknown product has further been determined by tandem mass spectrometry.

References:

1. C.C. Buckenmaier, E.H. Lee, C.H. Shields, J.B. Sampson, J.H. Chiles, Regional Anesthesia and Pain Medicine, 28: 321-327 (2003).

Comprehensive Serum Proteome Profiling Of Breast Cancer With The Application Of Multi-Dimensional Protein Identification Technology

Qinhua Cindy Ru[1,*], Luwang Andy Zhu[1], Yonghong Zhang[1], Jordan Silberman[1], Henry Brzeski[1], Michael Liebman[1], And Craig Shriver[2]

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Proteomic research and technology have recently undergone dramatic improvements, and these developments will soon lead to the discovery of biomarkers useful in the cancer early detection and diagnosis. Tools applied to this end include two-dimensional liquid chromatography coupled with tandem mass spectrometry (2-D LC-MS/MS), two-dimensional gel electrophoresis coupled with mass spectrometry (2-D gel/MS), and protein chip with surface-enhanced laser desorption and ionization (SELDI) mass spectrometry. Multi-dimensional protein identification technology (MudPIT), as a practical update of on-line 2-D LC-MS/MS technique, has recently been improved such that they provide better reproducibility, higher throughput, and greater mass spectrometry capabilities and become an effective alternative to two-dimensional gel electrophoresis (2-D gel). In the initial investigation of first one hundred serum samples including 78 breast cancer specimens (23 invasive, 14 atypical, and 41 benign) and 22 normal controls, five hundred twenty-six proteins were identified. When do the statistics on the identification frequency of proteins, twenty-four proteins were found only identified from breast cancer samples. Further investigation revealed that, within these twenty-four proteins, there was a subgroup containing four proteins has been identified in 87% of invasive samples and only 11% benign samples. The initial protein profiling investigation also exposed a few limitations existing in the current protein database strategies and hard to overcome. Therefore, a brand new method that does peptide profiling based on the peptide criteria acquired in 2-D LC-MS/MS analysis has been developed and applied in the peptide profiling of seventy breast cancer sera (13 atypical, 20 invasive, and 37 benign) and 19 normal controls.

Adventures In Biotechnology For High School Students:
A Biotech/Life Sciences Economic Development Initiative

Joan Schanck

Pittsburgh Tissue Engineering Initiative (PTEI)

Tissue engineering is at the forefront of modern medicine and the Pittsburgh Tissue Engineering Initiative (PTEI) has been at the forefront of tissue engineering for nearly a decade. The mission of PTEI is to improve the health of individuals by establishing the region as an internationally recognized center of

excellence in research, education, and commercial development for the advancement of tissue-related medical therapies.

In addition to participating in the Windber Research Institute's Showcase for Biotechnology 2005 as exhibitor and sponsor, PTEI is pleased to feature a component of one of our many educational and workforce development programs, *The Adventures in Biotechnology Program*. On Monday, August 15, 2005 students from Windber High School -- participants in the *Adventures in Biotechnology Program* -- who have formed a working partnership with researchers and leaders of the Windber Research Institute, will present their work to Congressman John P. Murtha.

ADVENTURES IN BIOTECHNOLOGY FOR HIGH SCHOOL STUDENTS:
A Biotech/Life Sciences Economic Development Initiative

Building on a world-class academic and medical research infrastructure and through the concerted effort of many regional stakeholders, the southwestern Pennsylvania region is expecting to create a biotech industry cluster of world prominence. To maintain this growth, and enhance the preparation of a workforce with relevant skills, PTEI and the Catalyst Connection have developed an *Adventures in Biotechnology Program* for high school students.

Adventures in Biotechnology is an eight-week working partnership between a team of students from a southwestern Pennsylvania high school and a biotech business or industry. The project provides high school juniors the opportunity to enhance their academic and technical skills by working directly with company employees to identify and solve business issues and/or design and build a product or to re-engineer an existing product, process, or system. The project utilizes leadership, support, communication, and accountability strategies in order to increase the academic, technical, and business skills of students in a work-based learning experience.

The ultimate goal of *Adventures in BioTechnology* is to build a sustainable, exemplary practice model that may be replicated throughout the Commonwealth, and to become an integrated component of school science and technology curriculum, providing direct linkage to business and industry leaders within the growing field of biotechnology.

From Bench To Bedside – An Integrated Approach To The Fight Against Breast Cancer

Mick Correll
Manager, Professional Services
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InforSense Ltd, working in collaboration with the Windber Research Institute is building a next generation medical informatics solution to bring the power of translational medicine to the fight against breast cancer. At the core of this solution is a patient-centric data warehouse that integrates a wide array of traditionally "silod" data sources. From genotypes to pathology, TET scans to proteomics, data from the clinic and the lab are made available to researchers and care providers alike. Along with disparate sources, are equally diverse communities of users. The creation and the web deployment of complex workflows enable informatics specialists to support the specific needs of various communities, while a common command and control center fosters collaboration across the groups. By starting with technology and techniques common in the domain of traditional business intelligence, and adapting it to provide the flexibility demanded by a rapidly evolving research field, our solution enables a powerful top down approach to research intelligence.

Genetic Analysis Of Complex Disease: Web-Based Management Tool For Dna Polymorphisms

Steven Ringquist

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Design of locus specific primers for use during genetic analysis requires the time consuming process of combining information from multiple sources when validating assays for large numbers of polymorphisms. Localizing freely available genetic databases, coupled with algorithm development for optimizing the generation of locus specific primers, aids assay development. Selection of Oligonucleotide Primers for PCR and Pyrosequencing (SOP3) software facilitates primer trio design by allowing the user to input gene locus symbols, SNP reference sequence numbers, or physical locations on the chromosome. SOP3 uses data found in dbSNP, automates the process of obtaining flanking sequence, and can limit the search of single nucleotide polymorphism (SNP) by incorporating information regarding haplotype and ethnicity for human polymorphisms. Validation attributes and locus-associated functional information for human and mouse polymorphisms are also incorporated. SOP3 output consists of oligonucleotide primers recommended for use in sequence-based genotyping. These data can be used for statistical tests evaluating the inheritance patterns of SNP markers. The software's data management and analysis modules allow it to be integrated as part of a laboratory organization suite. This work was supported by grants U19- AI056374-01 Autoimmunity Centers of Excellence, RO1DK24021 from the NIH, and ERHS #00021010 from the DOD.

Co-Occurrence Analysis And Directed Graph Visualization Of Diagnoses From Breast Pathology Slides

Sameer Soi, Susan Maskery, Craig Shriver, Jeffrey Hooke, Sean Guo, Michael Liebman, Hai Hu

Background: Heterogeneity in breast cancer pathology complicates diagnosis, staging, and treatment decisions for this disease. A systematic and quantitative study of heterogeneity in breast tissue would enable us to characterize the localized clustering of various pathologies, and use that characterization to guide further research into the complex pathologic associations within breast tissue.
Methods: This project focuses on breast pathology diagnoses that co-occur on a single slide. Our data set is drawn from patients participating in the Clinical Breast Care Project (CBCP) study between Windber Research Institute (WRI) and Walter Reed Army Medical Center (WRAMC). As part of the CBCP study, a large data warehouse was created to store all patient information collected at WRAMC and the results of all genomic and proteomic experiments done at WRI. We withdrew from the CBCP data warehouse the pathology diagnoses from 2463 breast pathology slides drawn from a population of 749 CBCP patients. There are 120 possible breast pathology diagnoses of which up to seven are recorded for a single slide.
Results and Discussion: With this data we set out to characterize the co-occurrence of multiple pathology diagnoses at the single slide level. An algorithm is developed to rank double diagnosis co-occurrence, then triple diagnosis co-occurrence, and finally quadruple diagnosis co-occurrence. R scripts are written to incorporate the results of the co-occurrence analysis in a tree data structure and for display in UDraw, a graph visualization software package. This type of quantitative study of breast tissue heterogeneity at the single slide level will help guide the molecular characterization of the complex pathologic associations within samples and between patients.

Proteomic Profiling Of Normal Human Urine Using Mudpit Analysis

Richard A. Katenhusen[1]*, Luwang (Andy) Zhu[1], Quinhua (Cindy) Ru[1], David Kirchner[1], Trevor J. Orchard[2], Darrell L. Ellsworth[1]
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Introduction:
The use of urine as a proteomic sample provides a wide array of disease applications and biomarker discovery. The abundance of urine from patients makes this sample type an ideal candidate for proteomic protein profiling. As with plasma and serum, urine is also plagued with high amounts of albumin along with other complications such as high abundance of salt and diluted protein concentrations. These challenges require a thorough concentration and separation method. Throughout the years there have been a variety of methods applied to the profiling of proteins in urine. Here we present a distinctive method for concentrating and desalting of human urine along with a MudPIT experiment resulting in the identification of over 100 unique proteins from normal human urine.
Method:
Urine was collected and frozen at –20C. Upon analysis sample was thawed at 37C followed by spinning at 3000xg for 10min. removing any cellular debris. 15ml aliquot of urine was added to a 20 ml 3000 MWCO. 5ml of 20% Acetonitrile was added to disrupt any protein- protein interactions and raised to a final volume of 20ml. Sample was concentrated to 5mls, then dH2O was added aiding in the removal of any remaining salts. Upon concentration to approximately 700ul the sample was transferred to a 1ml 3000MWCO. Further concentrating to an approximate volume of 100ul. Sample was then subjected to total trypsin digestion at 37C overnight. The resulting peptides were analyzed by MudPIT analysis by repetitively injecting the sample 10 times to increase protein identifications.

Ultrafiltration For The Identification Of Low Molecular Weight Proteins In Human Serum

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Serum is a rich source of biomarkers that reflects the pathophysiological condition of humans. The serum represents a spectrum of compounds that encompasses high abundant proteins, low abundant and low molecular weight proteins/peptides, protein fragments, carbohydrates, and lipids. We have been using a comprehensive approach to profile the serum molecular species of cancer specimens for quantitative and qualitative analysis to answer the causes of cancer at the molecular level. In the present study, our focus is to develop a rapid method to selectively fractionate low abundant and low molecular weight proteins from high molecular weight proteins. In this poster, we demonstrate the use of molecular weight cut off filters to separate the serum into fractions.

Pcr Amplification Of A Cpg Island In The Prdm15 Gene Prior To Developing A Hypermethylation Assay For Genes Deregulated In Breast Cancer

Michael Kaufman, Henry Brzeski, Tapan Maity, Michael Liebman
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Methylation is a form of genetic modification that is responsible for alterations in gene expression. Methylation is an essential process that takes place in X chromosome inactivation, imprinting, and the silencing of retroviral and transposable DNA elements. Methylation has also been found to cause silencing of tumor suppressor genes, which in turn promotes the onset of cancer. Methylation occurs in CpG islands which are typically found in the promoter regions of genes. These CpG islands are very rich in CpG dinucleotides. In normal adult human cells the cytosine of these CpG regions is usually found to be unmethylated, but in cancer it is typically in these CpG islands where the cytosine is found to be methylated. In general hypermethylated DNA sequences are often found to be less expressed or not expressed at all. In human cancer cells many tumor suppressor and other cancer related genes are found to be hypermethylated. Hypermethylation is thought to be involved in silencing these genes.

Primers have been generated using Primer3 which will isolate the specific CpG island we are looking at in the PRDM15 gene. PCR will be performed to isolate and amplify the CpG islands. Bisulfite reactions

from an EZ DNA methylation kit (Zymo Research) will be used to change the cytosines in methylated CpG islands to uracil, which eventually can be analyzed by DNA sequencing. Once this is accomplished the experiment will move to breast cancer tissue in order to identify other genes which are found to be hypermethylated.

CONCLUSIONS

Thirty-three research scientist and medical professionals from the United States and the United Kingdom presented lectures and poster sessions on a variety of research subjects in biomolecular research including DNA sequencing, genotyping, gene expression analysis, protein separation and identification, and biomedical informatics analysis. There were 27 booths demonstrating ongoing research programs, innovative emerging technologies, and the availability of commercial equipment and software. The meeting provided opportunities for and facilitated interdisciplinary formal and informal discussions which have lead to increased scientific collaborations in translational medicine. The meeting not only increased the knowledge among the scientific and medical communities of the research that is being performed at the Windber Research Institute, but it also increased the visibility of several other U.S. Army Medical Research and Materiel Command sponsored research programs. The conference highlighted the research being performed in the MRMC/TATRC supported Clinical Breast Care Project, Integrated Cardiac Healthcare Project, and Gynecological Disease Project. The conference also provided a community outreach program which recognized local high school students by inviting them to present their related research studies to the scientific audience. This effort enhanced the quality of science available to the students and faculty in this rural community and also introduced the participants to the availability of research opportunities in the Department of Defense and other federal and commercial organizations.

REFERENCES

N/A

APPENDICES

N/A